(ii) culturing said cells from the tissue of interest in vitro under permissive conditions such that the immortalizing function is activated; and

(iii) subjecting the cells to non-permissive conditions so as to result in a cessation of growth and in differentiation.

Please cancel claims 4, 8, 14, and 30-32.

REMARKS

Claims 1, 4, 7-9, 13 and 15-32 were pending. Claims 1,7, 9, 13, 17, 18, and 25 have been amended. Claims 4, 8, 14, and 30-32 have been cancelled. Following submission of this paper, claims 1, 7, 9, 13, and 15-29 are pending.

Support for the amendments can be found throughout the specification and in the claims as originally filed. No new matter has been added as a result of the amendments. A marked up of the claims indicating the changes made as a result of this amendment is attached.

The Objection to Claim 9 Should Be Withdrawn

The Examiner has objected to claim 9 under 37 C.F.R. § 1.75(c), as allegedly being of improper dependent form. Claim 9 has been amended to depend from claim 1, which is directed toward a cell line comprising a SU40 ts A58 gene. Applicants respectfully request withdrawal of the rejection.

The Claim Rejections Under 35 USC § 112 Should Be Withdrawn

Claims 1, 4, 7-9, 13, 15-32 have been rejected under 35 U.S.C. 112, ¶ 1. The Examiner alleges that specification "teaches only human neurofilament gene promoter NF-L, which is neuronal specific" and "does not provide support for any and all cell type specific promoters." (Office Action, p. 4)

Applicants respectfully traverse for the reasons stated in the Applicants' previously filed amendments. However, in order to further prosecution, independent claims 1, 7, 9, 13, 17, 18, and 25 have been amended to recite "human NF-L gene promoter." Claims 4, 8, 14, and 30-32 have been cancelled. In view of the foregoing, withdrawal of the rejection and prompt allowance of the claims are respectfully requested.

The Claim Rejections Under 35 USC § 103 Should Be Withdrawn

Claims 1, 4, 7-9, 13, 15-32 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Noble at al (WO 91/13150, 1991), Stocklin et al (J. Cell Bio. 12291):199-208, 1993), and Moses JH (Br. J. Cancer, 69(21):1, 1994) in view of Reeben et al (Biochem. Biophy. Res. Com. 192(2):465-470, 1993) in view of Leder et al (U.S. Pat No. 5,087,571) and further in view of Hammer et al (U.S. Pat. No. 5,489,742).

Applicants respectfully traverse. The claimed invention is not rendered obvious by the cited art using the objective standard for obviousness under 35 U.S.C. § 103(a). The Examiner has not provided *prima facie* evidence that one of ordinary skill in the art, using the teachings Noble, Stocklin, Moses, Yazdanbakhsh, Leder, and Hammer would be expected to be able to produce the claimed invention. (*See* M.P.E.P.

§ 2143.0 stating the prior art can be modified or combined to reject claims as *prima facie* obvious only so long as there is a reasonable expectation of success).

In particular, Applicants note that it is an unexpected result that the 6kbp fragment of the human NF-L promoter, when introduced into transgenic rats, as opposed to mice, would result in a completely unique tissue specific expression for the brain in the resultant transgenic rat. As explained in Reeben et al. ("Tissue-Specific Expression of Rat Light Neurofilamet Promoter-Driven Reporter Gene in Transgenic Mice" Biochem. & Biophysical Research Commun. vol: 192, pp. 465-70), "[p]reviously, several groups have studied NF-L promoter driven reporter genes or transient expression of complete NF-L genes in different cell lines, particularly in rat pheochromocytoma PC12 cells, C6 glioma cells, and fibroblasts. No cell-type specific expression was observed." Reeben at page 465 (emphasis added).

This language suggests that an NF-L promoter would not be useful for specific immortalization purposes using rat cells. Therefore, it is an unexpected result that the 6kbp fragment of the human NF-l promoter, when introduced into transgenic rats, as opposed to mice, would result in a completely unique tissue specific expression for the brain in the resultant transgenic rat. See Application, Table 2, page 28.

Moreover, the cited references neither suggest the claimed subject matter of the invention, nor do they provide a motivation to combine the references. Noble appears to be directed to the use of a promoter that can be regulated by an exogenous agent. Such a promoter is referred to as a "non-constitutive" promoter. The teaching of Noble is directed at constructs and transgenic animals in which immortalization of cell

lines can be easily controlled by external factors. Broad specificity promoters that are controlled by specific external signals are preferred as they allow cell lines to be derived for a wide variety of tissues.

In contrast, the object of the present invention is to provide a construct which preserves the differentiation specific features of particular cell type, in particular neuronal cells. There are few alternatives available that can provide large quantities of differentiated neuronal tissue *in vitro* for experimental work. The NF-L promoter disclosed in the present invention does not fall under the definition of the "non-constitutive" promoter of Noble in that it is necessarily expressed in neuronal cell lines to product the differentiated phenotype and is not activated by exogenous signals. Thus, the use of the NF-L promoter teaches away from the disclosure of Noble despite being coupled to a conditional immortalizing gene, since the promoter can maintain the desired features of differentiation in cell lines produced.

Similarly, neither Moses nor Stocklin teach a cell type specific promoter. As stated in Stocklin p.201, "[d]espite the implication in its name, the MMTV LTR is not exclusively active in mammary gland cells. High level of expression have been detected in mammary epithelium, but expression was also found in the epithelial cells of salivary glands, lung, kidney, seminal vesicles, testes, and also in lymphoid cells in spleen and thymus." (Emphasis added). Further, the expression in Stocklin et al. is not tissue specific since expression of the transgene was observed in the kidney, lung, mammary gland, salivary gland, and in the epithelial cells of the male reproductive tract.

Yazdanbakhsh does not cure the defect. It was not obvious when the applicants' NF-L construct was made that it would be expressed in precursor cells to product sufficient large T-Antigen to immortalize cells at the permissive temperature of the tsA58-SV40 gene because the NF-L promoter was thought only to be active in postmitotic neurons. (*See* Reeben et al., Biochem. Biophys Res Commu. Vol:192, pp. 465-70, Tissue-Specific Expression of Rat Light Neurofilament Promoter-Driven Reporter Gene in Transgenic Mice," 1993). Expression from this promoter has not previously been observed in either embryonic stem cells or neuronal precursors. Thus, prior to the instant invention, it was believed that for an NF-L promoter to immortalize cells would either require de-differentiation of the cells reverting to a state similar to a stem cell, or that there is a population of neuronal stem cells that are isolated by the methods of this invention.

The Examiner has cited no evidence that anyone has produced a transgenic animal with a neuronal cell specific promoter operably linked to a conditional oncogene, transforming gene, immortalizing gene, or a cell cycle affecting gene.

In addition, none of the prior art references teach or suggest a neuronal cell line derived from a rat comprising a conditional oncogene, transforming gene, immortalizing gene or cell cycle affecting gene operably linked to a cell type specific promoter, particularly a human NF-L promoter.

The fact that rats are widely used in biomedical research does not provide the requisite motivation to generate a neuronal cell line from a rat having a human NF-L promoter.

Therefore, applicants submit that the claims are patentable and respectfully request withdrawal of the rejection.

CONCLUSION

In view of the foregoing, applicants respectfully submit that the application is condition for allowance and earnestly request prompt allowance of the claims. Authorization to charge the deposit account for payment of the three month extension of time fee is submitted herewith.

Respectfully submitted,
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MARKED UP VERSION OF THE CLAIMS TO INDICATE THE CHANGES MADE AS A RESULT OF THIS AMENDMENT

1. (Fourth Amendment) A neuronal cell line obtained from a transgenic rat, the cells of which comprise:

(i) a conditional oncogene, transforming gene or immortalizing gene or a cell cycle affecting gene operably linked to

(ii) a cell type specific promoter ,

in which the conditional oncogene, transforming gene or immortalizing gene or the cell cycle affecting gene is a SV40tsA58 gene

and in which the cell type specific promoter is a human NF-L gene promoter.

7. (Fourth Amendment) A neuronal cell line obtained from a transgenic rat, the cells of which comprise:

(i) a conditional oncogene, transforming gene or immortalizing gene or a cell cycle affecting gene operably linked to

(ii) a cell type specific promoter

[and] in which the conditional oncogene, transforming gene, immortalizing gene or the cell cycle affecting gene is a C Erb β2 gene or a TGF α gene

and in which the cell type specific promoter is a human NF-L gene promoter.

9. (Amended) A cell line as claimed in claim [8] 1 having the

ECACC Accession number 96092454.

13. (Thrice Amended) A method of producing a transgenic rat, comprising:

(i) causing a female rat to super-ovulate by supplying her with a regular supply of Follicle Stimulating Hormone (FSH) prior to mating;

(ii) mating or artificially inseminating the female rat;

(iii) obtaining the resulting embryo from the female rat; and

(iv) incorporating

(i) a conditional oncogene, transforming gene or immortalizing gene or a cell cycle affecting gene operably linked to

(ii) a cell specific promoter into the genome of the rat embryo

in which the conditional oncogene, transforming gene or immortalizing gene or the cell cycle affecting gene is a SV40tsA58 gene, C Erb β2 gene or a TGF α gene

and in which the cell type specific promoter is a human NF-L gene promoter.

17. (Thrice Amended) A transgenic rat whose germ cells and somatic cells contain

(i) a conditional oncogene, transforming gene or immortalizing gene or a cell cycle affecting gene operably linked to

(ii) a cell type specific promoter as a result of chromosomal

incorporation into the rat genome or into the genome of an ancestor of said rat

in which the conditional oncogene, transforming gene or immortalizing gene or the cell cycle affecting gene is a SV40tsA58 gene

and in which the cell type specific promoter is a human NF-L gene promoter.

18. (Thrice Amended) A transgenic rat whose germ cells and somatic cells contain

(i) a conditional oncogene, transforming gene or immortalising gene or a cell cycle affecting gene operably linked to

(ii) a cell type specific promoter as a result of chromosomal incorporation into the rat genome or into the genome of an ancestor of said rat,

wherein the conditional oncogene, transforming gene, immortalising gene, or the cell cycle affecting gene is a C Erb β 2 gene or a TGF α gene

and wherein the cell type specific promoter is a human NF-L gene promoter.

25. (Twice Amended) A method of generating a cell line from a transgenic rat comprising a conditional oncogene, transforming gene or immortalizing gene or a cell cycle affecting gene operably linked to a cell specific promoter wherein the cell type specific promoter is a human NF-L gene promoter, the method comprising:

(i) maintaining the rat at restrictive conditions such that the

conditional oncogene, transforming gene or immortalizing gene or the cell cycle affecting gene is a SV40tsA58 gene, a C Erb β 2 gene, or a TGF α gene and is expressed in vivo, only in a tissue of interest and in an inactive form such that the cells thereof grow normally;

(ii) culturing said cells from the tissue of interest in vitro under permissive conditions such that the immortalizing function is activated; and

(iii) subjecting the cells to non-permissive conditions so as to result in a cessation of growth and in differentiation.